ANIMAL MODELS OF BRAIN TUMORS

Part 2. Animal Models Offer Insights into Human Brain Neoplasms

Pituitary Models

Walavan Sivakumar, M.D. and William T. Couldwell, M.D., Ph.D.

Department of Neurosurgery, Clinical Neurosciences Center, University of Utah, Salt Lake City, Utah

Corresponding author: William T. Couldwell, M.D., Ph.D.

Department of Neurosurgery

Clinical Neurosciences Center

University of Utah

175 N. Medical Drive East

Salt Lake City, UT 84132

Phone: 801-581-6908

Fax: 801-581-4385

Email: neuropub@hsc.utah.edu

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Summary

Pituitary tumor animal models provide researchers a microenvironment that simulates the clinical situation; however, in comparison with astrocytoma and meningioma tumor research where intracranial xenograft transplantations are increasingly being used to test various therapeutic modalities, *in vivo* therapeutic research on pituitary animal models focuses on direct drug therapy to the tumor because of the lack of established intracranial pituitary tumor models. The rat subcutaneous prolactin-secreting pituitary model allows investigators to noninvasively measure tumor size and the effect of direct tumor-guided therapy in a serial manner and is considered biologically relevant because it has proven to be histologically, immunocytochemically, and ultrastructurally consistent with human pituitary tumors.

Key Words

Pituitary tumor; pituitary adenoma; adenohypophysis; neurohypophysis

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Animal models of pituitary tumors

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1. Introduction

Pituitary tumors are a broad group that accounts for approximately 15% of all symptomatic adult primary intracranial tumors. They are remarkably prevalent, present in approximately 20% of the population (*1*). Most of these tumors are small and incidental, but roughly 1 patient in 600–700 requires treatment (*2*).

Pituitary tumors can be classified by multiple different schemes, including by endocrine function, by histological staining methods, and by electron microscopic appearance (*3*). The mean age of affected patients is between 30 and 50 years. There is a slight female predominance with prolactin- and adrenocorticotropic hormone–secreting tumors and a male predominance in growth hormone–secreting tumors. Nonfunctioning pituitary adenomas account for 25% of these tumors and less than 1% are malignant.

Animal tumor model systems are vital to the process of cancer therapy development (*4*). Pituitary tumor animal models provide researchers a microenvironment that simulates the clinical situation. Since 1953 when Furth et al. (*5*) described transplantable pituitary tumors in the rat, numerous constructs and induction methods enlisting mouse, rat, and canine models have been described (*6-9*). In comparison with astrocytoma and meningioma tumor research where intracranial xenograft transplantations are increasingly being used to test various therapeutic modalities, *in vivo* therapeutic research on pituitary animal models focuses on direct drug therapy to the tumor because of the lack of established intracranial pituitary tumor models.

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1.1. History of Pituitary Tumor Models

Early pituitary tumors in animal models were induced by exposing rats to elevated levels of estrogen (*10*). Other methods of inducing tumors included antithyroid procedures, carcinogen exposure, and increasing levels of ionized radiation (*11*). Further research with the estrogen-induced tumors showed that these tumors were completely hormone dependent and not autonomous neoplasms, making them only viable in estrogen-treated hosts (*8, 12, 13*). Additionally, investigators found that these inducible tumors that were transplanted into other animals differed from most human pituitary tumors by their malignancy and their undifferentiated aspects, reducing their value as a human pituitary neoplasm model (*14, 15*).

In 1982, Trouillas *et al.* described spontaneous tumors that arose in the pituitary of approximately 70% of all female Wister/Furth rat strains (*16, 17*). Histological, immunocytochemical, and ultrastructural similarities between these spontaneous tumors and those found in the human pituitary made this entity a very promising animal tumor model for study (*16*). Initial difficulties using this model included the length of time required to mature enough animals with acceptable tumors, the small size of the tumors, and the unusual location, making it a difficult and costly model for pituitary study. By grafting these spontaneous tumors directly under the kidney capsule and subcutaneous region of consanguineous animals within the same strain, however, investigators developed an animal model of the pituitary tumor that was easy to transplant into experimental groups, effectively

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reproducible, and analogous to the human pituitary tumor. This initial cell strain was named the spontaneous mammotrophic tumor in Wistar (SMtTW) rats line (*8*). Since that time, other pituitary tumor cell lines that have been shown to have similar morphological and functional properties as the SMtTW line include the ArT and GH strains.

The rat subcutaneous prolactin-secreting pituitary model allows investigators to noninvasively measure tumor size and the effect of direct tumor-guided therapy in a serial manner. The results from these studies are considered biologically relevant as these animal models have been proved to be histologically, immunocytochemically, and ultrastructurally consistent with human pituitary tumors (*18, 19*). The majority of our work has been done with the GH pituitary tumor cell line, namely the GH_4C_1 strain.

2. Materials

The products and suppliers used are listed below. Comparable products should also be effective.

2.1. Pituitary tumor cell culture

1. Rat pituitary cell line, such as GH_4C_1, grown at 37°C in 5% CO_2
2. Growth medium: Ham's F-10 Nutrient, supplemented with 15% horse and 2.5% bovine serum in the absence of antibiotics
3. 10-cm cell culture dishes
4. T-175 flasks

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2.2 Tumor cell implantation and tumor size measurement

1. GH4C1 cell line at ~80% confluence in 10-cm dishes (maximum of 9 weekly passages)
2. Ice
3. 0.025% Trypsin
4. Hemocytometer
5. Growth medium: Ham's F-10 Nutrient, supplemented with 15% horse and2.5% bovine serum in the absence of antibiotics
6. 8-week-old female Wistar-Furth rats
7. Intraperitoneal injections of 100 mg/kg ketamine and 10 mg/kg xylazine hydrochloride (Sigma) in phosphate-buffered saline
8. 21-gauge needle
9. Syringe
10. Calipers

3. Methods

3.1. *Pituitary tumor cell culture*

1. Grow the GH4C1 tumor cell lines in Ham's F-10 Nutrient supplemented with 15% horse and 2.5% bovine serum in the absence of antibiotics to 80% confluence in cell culture dishes at 37°C and 5% CO_2 (*see* **Note 1**).

3.2. Tumor cell implantation and tumor size measurement

1. Grow cells to approximately 80% confluence in T-175 flasks.
2. All steps are carried out on ice.
3. Treat cells with 0.025% trypsin to detach cells from flasks.
4. Determine cell counts using a bright-light hemocytometer; centrifuge the cells at 1000 rpm for 5 minutes at 4°C and resuspend the pellet in medium to obtain final cell concentrations of 10^7 cells per rat in 100 μl of growth medium (see Note 2).
5. Anesthetize 8-week-old Wistar-Furth rats with intraperitoneal injections of 100 mg/kg ketamine and 10 mg/kg xylazine hydrochloride in phosphate-buffered saline (see Note 3).
6. Inoculate approximately 1×10^7 GH4C1 cells subcutaneously between the scapulae of each rat.
7. After implantation, allow tumors to grow in vivo for 28-day period.
8. After 28-day growth period, determine tumor sizes using surface diameter measurements with calipers in three orthogonal directions.
9. Subsequently, animals can be equally divided so that each group has tumors of comparable sizes (see Note 4).
10. Give animals open access to food, water, and evaluate tumor size three times weekly.
11. Sacrifice animals that appear ill or with neurological deficits in accordance with the animal protocol at your institution (see Note 5).
12. Harvest tumors from all animals at time of death for paraffin blocks and electron microscopy.

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4. Notes

1. Stock cultures are grown on cell culture plates before being transplanted to T-175 flasks for further passaging. GH4C1 tumor cell line quality begins to diminish in regards to replication ability after 10 passages. Therefore, we recommend only using cell lines that have undergone a maximum of 9 passages.
2. For subcutaneous tumor inoculations in the rat model, we found 10^7 cells per animal to be ideal because of easier visibility of tumor and longer time before tumor-related side effects.
3. Wistar-Furth rats should be allowed to acclimatize for one week prior to tumor cell injection. Follow your Institutional Animal Care and Use Committee protocols regarding all animal storage and handling.
4. To minimizing fighting between rats and risk of bodily damage to animals or tumor specimens, we use only female rats in our protocol and follow institutional guidelines regarding the maximum number of animals per cage.
5. While it did not occur in our series, all animals, per institutional care protocols, that appear ill or show neurologic deficits, should be euthanized.

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6. References

1. Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, and McCutcheon IE (2004) The prevalence of pituitary adenomas: a systematic review, Cancer **101**: 613-619.
2. Couldwell WT, and Cannon-Albright L (2010) A heritable predisposition to pituitary tumors, Pituitary **13**: 130-137.
3. DeLellis R, Lloyd R, Heitz P, and Eng C, (Eds.) (2004) *Pathology and Genetics of Tumours of Endocrine Organs (IARC WHO Classification of Tumours)*, IARC Press, Lyon.
4. Khleif SN, (Ed.) (2003) *Animal Models in Developmental Therapeutics*, Vol. 2, 6 ed., BC Decker, Hamilton.
5. Furth J, Gadsen EL, and Upton AC (1953) ACTH secreting transplantable pituitary tumors, Proc Soc Exp Biol Med **84**: 253-254.
6. Lloyd RV (1991) Ultrastructure of spontaneous and transplanted pituitary tumors in laboratory animals, J Electron Microsc Tech **19**: 64-79.
7. Ito A (1976) Animal model of human disease: pituitary tumors, Am J Pathol **83**: 423-426.
8. Trouillas J, Girod C, Claustrat B, Joly-Pharaboz MO, and Chevallier P (1990) Spontaneous prolactin transplantable tumor in the Wistar/Furth rat (SMtTW): a new animal model of human prolactinoma, Cancer Res **50**: 4081-4086.
9. El Etreby MF, Muller-Peddinghaus R, Bhargava AS, and Trautwein G (1980) Functional morphology of spontaneous hyperplastic and neoplastic lesions in the canine pituitary gland, Vet Pathol **17**: 109-122.

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10. Lloyd RV (1983) Estrogen-induced hyperplasia and neoplasia in the rat anterior pituitary gland. An immunohistochemical study, Am J Pathol **113**: 198-206.

11. Yokoro K IA (1981) *Experiemental Pituitary Tumors*, Springer-Verlag, Berlin.

12. Treip C (1961) Pathology of human and experimental pituitary tumours, Proc R Soc Med **54**: 623-627.

13. Treip CS (1983) The regression of oestradiol-induced pituitary tumours in the rat, J Pathol **141**: 29-40.

14. Ito A, Furth J, and Moy P (1972) Growth hormone-secreting variants of a mammotropic tumor, Cancer Res **32**: 48-56.

15. Lamberts SW, and MacLeod RM (1979) The inability of bromocriptine to inhibit prolactin secretion by transplantable rat pituitary tumors: observations on the mechanism and dynamics of the autofeedback regulation of prolactin secretion, Endocrinology **104**: 65-70.

16. Trouillas J, Girod C, Claustrat B, Cure M, and Dubois MP (1982) Spontaneous pituitary tumors in the Wistar/Furth/Ico rat strain. An animal model of human prolactin adenoma, Am J Pathol **109**: 57-70.

17. Prysor-Jones RA, and Jenkins JS (1981) Effect of bromocriptine on DNA synthesis, growth and hormone secretion of spontaneous pituitary tumours in the rat, J Endocrinol **88**: 463-469.

18. Cole CD, Liu JK, Sheng X, Chin SS, Schmidt MH, Weiss MH, and Couldwell WT (2008) Hypericin-mediated photodynamic therapy of pituitary tumors: preclinical study in a GH4C1 rat tumor model, J Neurooncol **87**: 255-261.

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19. Hamilton HB, Hinton DR, Law RE, Gopalakrishna R, Su YZ, Chen ZH, Weiss MH, and Couldwell WT (1996) Inhibition of cellular growth and induction of apoptosis in pituitary adenoma cell lines by the protein kinase C inhibitor hypericin: potential therapeutic application, J Neurosurg **85**: 329-334.

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